Electronic Supplementary Material (ESI) for Metallomics

Thermodynamic and spectroscopic investigation on the role of Met residues in Cu^{II} binding to the non-octarepeat site of the human Prion protein

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SUPPLEMENTARY INFORMATION

Tab. S1 - Spectroscopic parameters of Cu(II) complexes at 25 °C in aqueous solution.

Main species	pH	UV/Vis		CD		EPR	
		λ_{max}/nm	ε/ M^{-1} cm^{-1}	λ/nm	Δε/M^{-1} cm^{-1}	A_{II} /G	g_{II}
hPrP106-113[(a)]							
Cu^{2+}, $[CuLH_2]^{4+}$	**5.0**	789	18	260	+0.12	120	2.41
$[CuL]^{2+}$	**6.5**	614	86	255	+2.19	163	2.23
				329	-0.17		
				532	+0.11		
				615	-0.01		
$[CuL]^{2+}$	**7.5**	607*	87*	256	+2.25	164	2.22
$[CuLH_{-1}]^{+}$				308 (sh)	+0.08		
				344	-0.05		
				392	+0.02		
				484	-0.09		
				557 (sh)	+0.04		
				633	+0.10		
$[CuLH_{-1}]^{+}$	**8.5**	569	82	256	+2.27	179	2.20
				316	+0.31		
				367	-0.03		
				493	-0.28		
				628	0.25		
$[CuLH_{-1}]^{+}$	**9.0**	534	105	257	+2.29	183	2.19
				316	+0.38		
				360	-0.03		
				494	-0.32		
				626	+0.29		
$[CuLH_{-2}]$	**10.0**	524	105	257	+2.32	182	2.19
				316	+0.42		
				362	-0.02		
				494	-0.36		
				627	+0.31		
$[CuLH_{-3}]^{-}$	**11.0**	532	107	257	+2.37	184	2.19
				318	+0.42		
				494	-0.39		
				628	+0.33		
(M109n-Leu)hPrP106-113							
Cu^{2+}, $[CuLH_2]^{4+}$	**5.5**	n. d.	n. d.	254	+0.59	118	2.42
				343	-0.06	133	2.36
				525	+0.06		
$[CuL]^{2+}$	**6.6**	n. d.	n. d.	251	+8.51	169	2.22
				346	-0.75		
				531	+0.50		
				627	-0.14		
$[CuL]^{2+}$	**7.0**	n. d.	n. d.	251	+9.29	173	2.30
				346	-0.75		
				535	+0.46		
				628	-0.05		
$[CuLH_{-1}]^{+}$	**8.5**	n. d.	n. d.	256	+8.64	194	2.19
				3.13	+1.06		
				355	-0.30		
				491	-1.00		
				628	+0.88		
$[CuLH_{-1}]^{+}$	**9.0**	n. d.	n. d.	257	+8.60	195	2.20
				314	+1.26		
				357	-0.23		
				491	-1.22		
				628	+1.05		
$[CuLH_{-2}]$	**10.0**	n. d.	n. d.	258	+8.57	195	2.19
				315	+1.36		
				358	-0.19		
				492	-1.35		
				628	+1.15		
$[CuLH_{-3}]^{-}$	**11.0**	n. d.	n. d.	257	+8.73	194	2.19
				316	+1.33		

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				360	-0.16		
				493	-1.43		
				629	+1.21		
(M109,112n-Leu)hPrP106-113							
Cu^{2+}, $[CuLH_2]^{4+}$	**5.5**	705	26	252	+3.66	118	2.42
				346	-0.37	129	2.36
				523	+0.23		
				620	-0.08		
$[CuLH_2]^{4+}$	**6.0**	605	73	250	+9.10	119	2.41
$[CuLH]^{3+}$				347	-0.80	131	2.36
$[CuL]^{2+}$				527	+0.39		
				613	-0.07		
$[CuL]^{2+}$	**7.0**	595	112	254	+8.86	166	2.23
				308 (sh)	+0.88		
				351	-0.51		
				485	-0.47		
				629	+0.47		
$[CuL]^{2+}$	**8.0**	535	151	257	+8.37	171	2.22
$[CuLH_{-1}]^{+}$				314	+1.30		
				358	-0.26		
				491	-1.10		
				627	+0.97		
$[CuLH_{-1}]^{+}$	**9.0**	534	166	257	+8.51	187	2.20
				316	+1.33		
				359	-0.18		
				494	-1.28		
				630	+1.13		
$[CuLH_{-2}]$	**10.5**	528	177	258	+8.69	190	2.20
				317	+1.36		
				360	-0.17		
				494	-1.34		
				630	+1.15		
Ac-HMAGAA-NH_2 [(b)]							
$[CuL]^{2+}$	**6.0**	760	17	220	4.44	159	2.31
				225	-1.35		
				260	0.03		
$[CuLH_{-2}]$	**8.0**	623	81	250	2.69	177	2.21
				301	-0.25		
				340	0.08		
				610	-0.51		
$[CuLH_{-3}]^{-}$	**10.0**	565	100	252	6.86	187	2.19
				303 (sh)	0.16		
				340	-0.54		
				512	-0.48		
				664	-0.25		
$[CuLH_{-4}]^{2-}$	**11.0**	538	107	251	6.47	191	2.19
				303 (sh)	0.07		
				340	-0.52		
				520	-0.49		
				658	-0.24		
Ac-HMAGA-NH_2							
$[CuL]^{2+}$	**6.0**	740	27	n. d.	n. d.	n. d.	n. d.
$[CuLH_{-2}]$	**8.0**	625	90	n. d.	n. d.	n. d.	n. d.
$[CuLH_{-3}]^{-}$	**11.0**	538	118	n. d.	n. d.	n. d.	n. d.
Ac-H(n-L)AGA-NH_2							
$[CuL]^{2+}$	**6.5**	723	29	251	0.13	120	2.41
				315	-0.02	128	2.36
				602	-0.04		
$[CuLH_{-2}]$	**8.0**	646	64	252	0.82	152	2.24
				308	-0.06	170	2.27
				350	0.03		
				604	-0.22		
$[CuLH_{-3}]^{-}$	**10.0**	520	81	254	5.41	155	2.23
		592 (sh)	-	300 (sh)	1.01		
				343	-0.43		
				568	-0.42		
$[CuLH_{-4}]^{2-}$	**11.0**	520	105	253	5.47	195	2.19
				300 (sh)	1.07		

				343	-0.43		
				494	-0.56		
				613	-0.17		
Ac-HMAAA-NH$_2$							
$[CuL]^{2+}$	**6.5**	666	41	255	0.21	117	2.42
				317	-0.04	128	2.36
				377	0.03		
				599	-0.08		
$[CuLH_{-2}]$	**8.0**	633	96	253	1.59	149	2.24
				305	-0.16		
				340	0.11		
				604	-0.61		
$[CuLH_{-3}]^{-}$	**10.0**	604	117	250	3.49	153	2.23
				320	0.29		
				523	-0.58		
				615	-0.55		
$[CuLH_{-4}]^{2-}$	**11.0**	530	159	242	6.04	202	2.18
				281	-0.98		
				309	0.71		
				502	-2.30		
				591	0.43		
Ac-HAMAA-NH$_2$							
$[CuL]^{2+}$	**6.5**	688	38	252	0.26	116	2.42
				316	-0.13	128	2.36
				372	0.05		
				610	-0.10		
$[CuLH_{-2}]$	**8.5**	635	120	251	1.53	157	2.24
				314	-0.42		
				368	0.24		
				607	-0.52		
$[CuLH_{-4}]^{2-}$	**11.0**	540	158	245	4.60	194	2.19
				290	-0.15		
				315	0.14		
				355	-0.03		
				510	-1.21		
Ac-HAAMA-NH$_2$							
$[CuL]^{2+}$	**6.5**	686	31	252	0.27	117*	2.42*
				306	-0.04	128*	2.36*
				607	-0.06		
$[CuLH_{-2}]$	**8.0**	637	72	251	1.51	147	2.24
				310	-0.22	173	2.28
				348	0.08		
				611	-0.37		
$[CuLH_{-4}]^{2-}$	**11.0**	529	137	243	6.19	196	2.19
				284	-0.77		
				314	0.28		
				510	-1.58		
Ac-HAAAM-NH$_2$							
$[CuL]^{2+}$	**6.5**	680	38	250	0.34	118	2.42
				307	-0.08	133	2.28
				345	0.04		
				602	-0.10		
$[CuLH_{-2}]$	**8.5**	631	108	251	1.86	145	2.24
				306	-0.33		
				345	0.24		
				608	-0.57		
$[CuLH_{-4}]^{2-}$	**11.0**	526	172	244	5.43	200	2.19
				286	-0.44		
				311	0.32		
				511	-1.61		

[a] Data from M. Remelli, M. Donatoni, R. Guerrini, A. Janicka, P. Pretegiani and H. Kozlowski, Dalton Tran. 2005, 2876-2885.

[b] Data from E. Gralka, D. Valensin, E. Porciatti, C. Gajda, E. Gaggelli, G. Valensin, W. Kamysz, R. Nadolny, R. Guerrini, D. Bacco, M. Remelli and H. Kozlowski, Dalton Trans. 2008, 5207-5219

* pH = 7.0

n. d. = not determined

Fig. S1 – Distribution diagram for the system Cu^{II}/ $hPrP_{106-113}$ at T = 298.2 K and I = 0.1 M. $[Cu^{II}]_{tot}$ = 0.1 mM; $[L]_{tot}$ = 1.0 mM

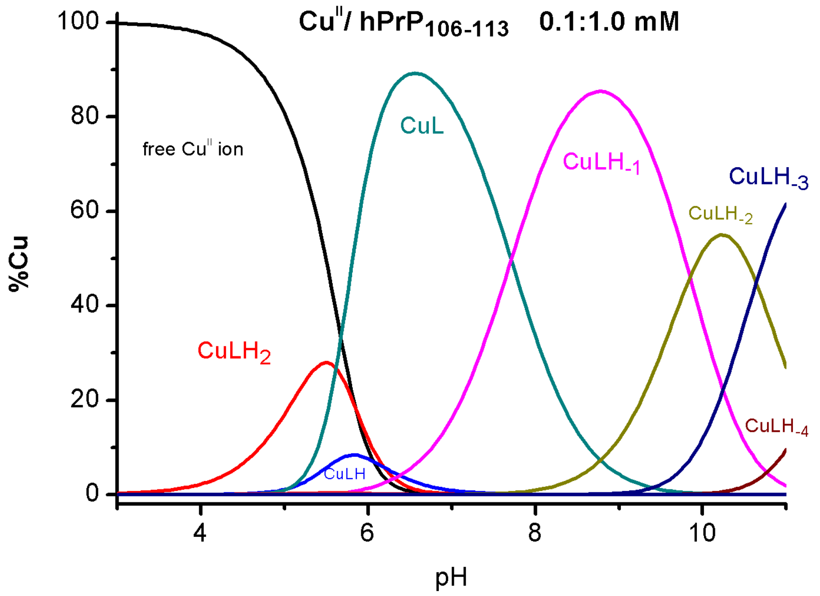

Fig. S2 – Distribution diagram for the system Cu^{II}/ $hPrP_{106-113}$ at T = 298.2 K and I = 0.1 M. $[Cu^{II}]_{tot}$ = 0.2 mM; $[L]_{tot}$ = 1.0 mM

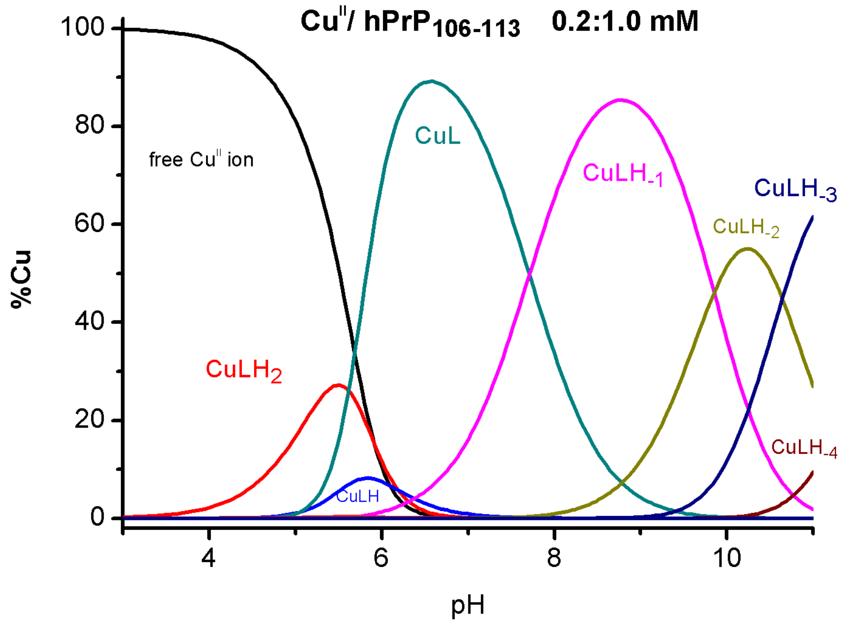

Fig. S3 – Selective region of 2D NMR ^{1}H-^{1}H TOCSY spectra (M112*n*-Leu)hPrP$_{106-113}$ 1 mM, pH 6.5, T 298 K, in the absence (black contours) and in the presence of 0.2 Cu(II) equivalents (green contours).

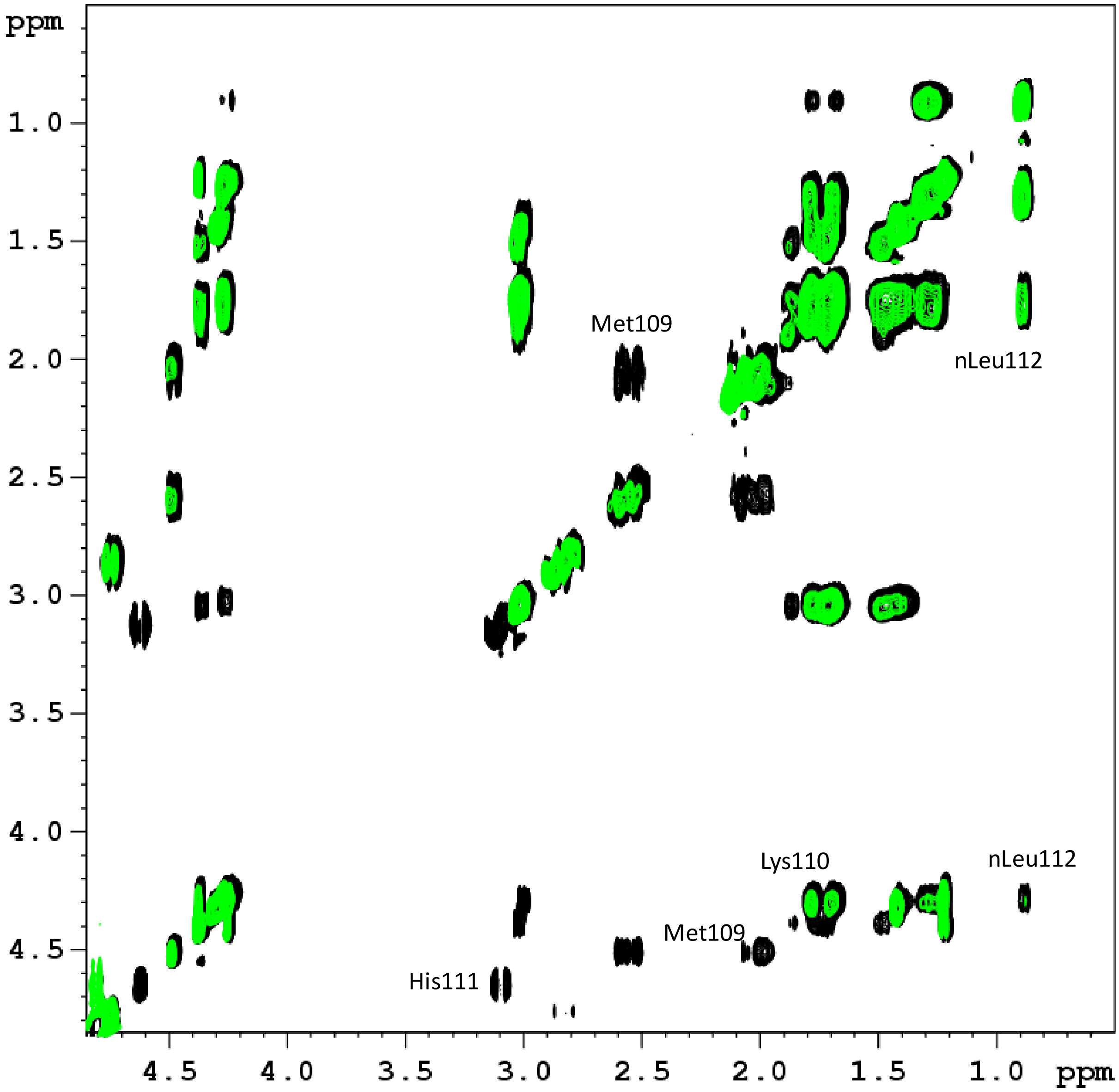

Fig. S4 – Distribution diagram for the system Cu^{II}/ $hPrP_{106-113}$ at T = 298.2 K and I = 0.1 M. $[Cu^{II}]_{tot}$ = 0.9 mM; $[L]_{tot}$ = 1.0 mM

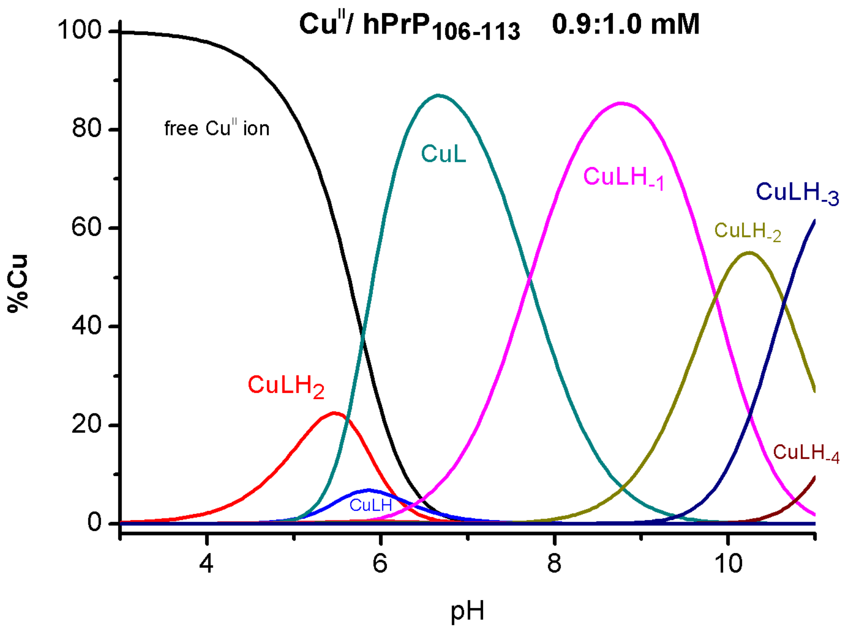

Fig. S5 – Experimental thermogram obtained by isoperibolic calorimetry, titrating an alkaline solution of Cu^{II}/ Ac-H(*n*-L)AGA with standard HNO_3.
T = 298.2 K and I = 0.1 M. $[Cu^{II}]_{tot}$ = 1.3 mM; $[L]_{tot}$ = 1.4 mM

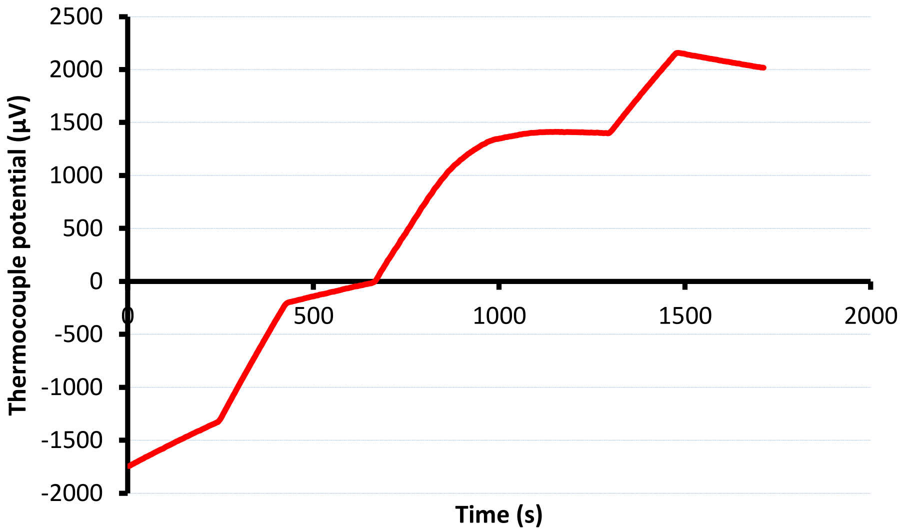

Fig. S6a - CD spectra for the system Cu^{II} / (M109*n*-Leu)$PrP_{106-113}$, at T = 298.2 K. $[Cu^{II}]_{tot}$ = 2.0 mM; $[L]_{tot}$ = 2.0 mM.

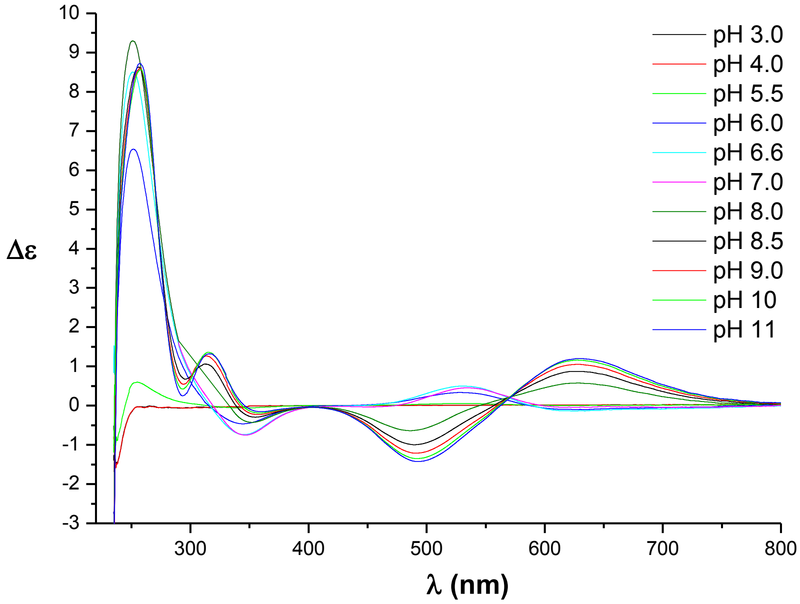

Fig. S6b - EPR spectra for the system Cu^{II} / (M109*n*-Leu)$PrP_{106-113}$. $[Cu^{II}]_{tot}$ = 2.0 mM; $[L]_{tot}$ = 2.0 mM.

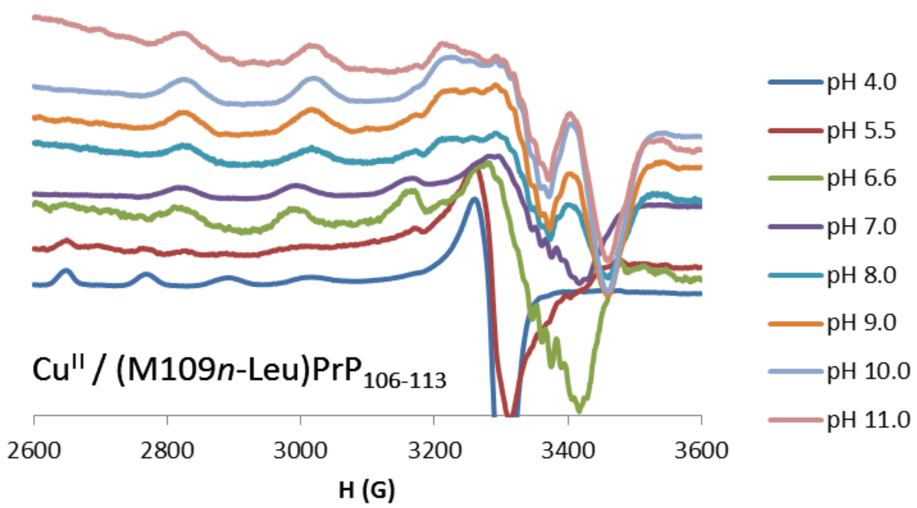

Fig. S7a - CD spectra for the system Cu^{II} / (M109,112*n*-Leu)PrP$_{106-113}$, at *T* = 298.2 K. $[Cu^{II}]_{tot}$ = 1.5 mM; $[L]_{tot}$ = 1.5 mM.

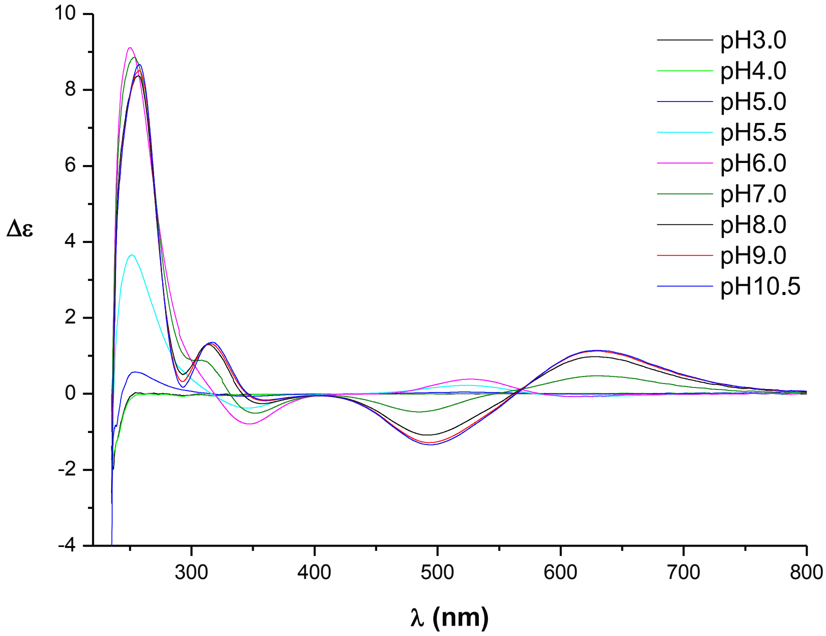

Fig. S7b – EPR spectra for the system Cu^{II} / (M109,112*n*-Leu)PrP$_{106-113}$. $[Cu^{II}]_{tot}$ = 1.0 mM; $[L]_{tot}$ = 1.0 mM.

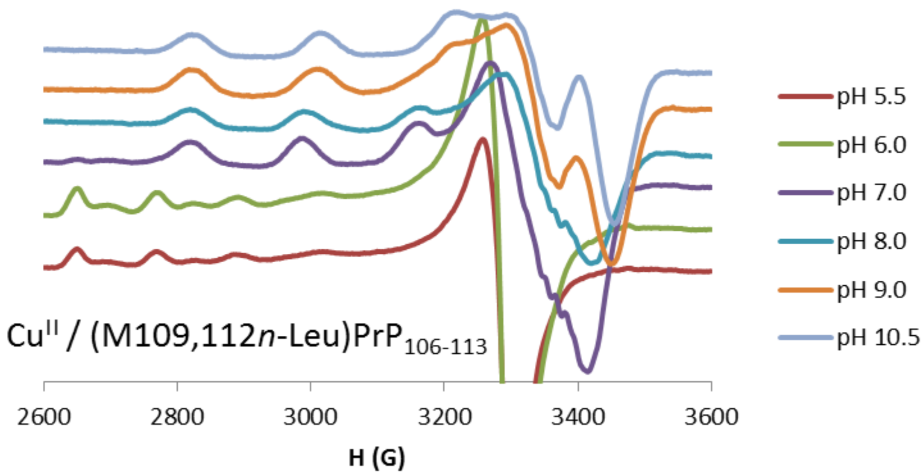

Fig. S8a - CD spectra for the system Cu^{II} / Ac-H(*n*-L)AGA, at *T* = 298.2 K.
$[Cu^{II}]_{tot}$ = 1.0 mM; $[L]_{tot}$ = 1.0 mM.

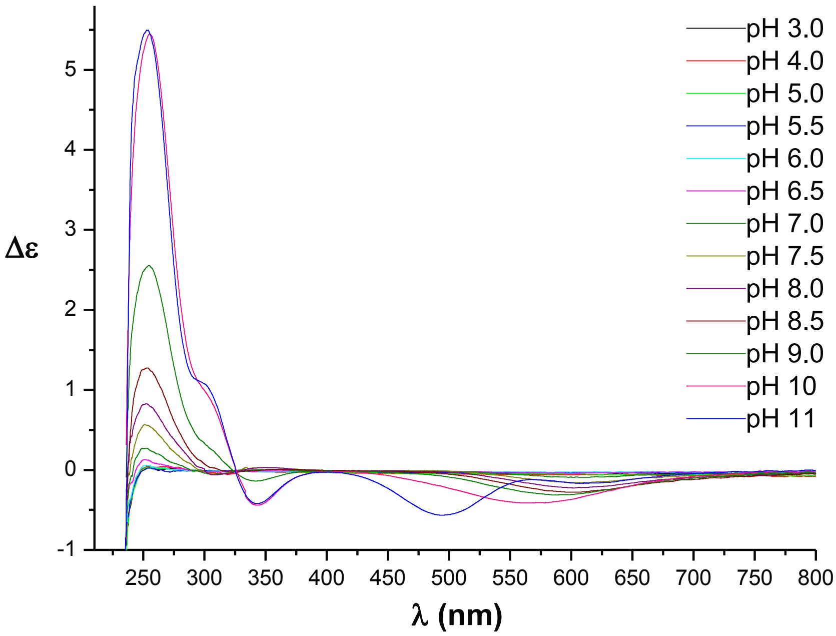

Fig. S8b - EPR spectra for the system Cu^{II} / Ac-H(*n*-L)AGA.
$[Cu^{II}]_{tot}$ = 1.0 mM; $[L]_{tot}$ = 1.0 mM.

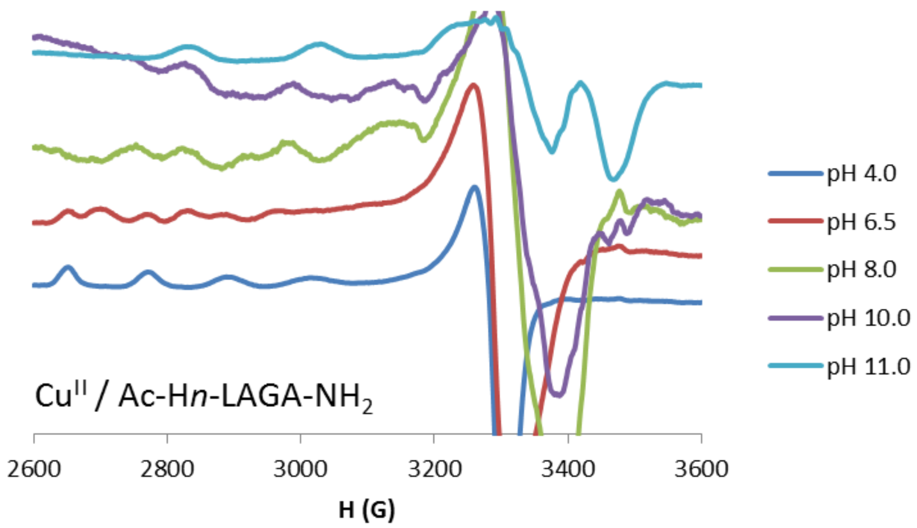

Fig. S9a - CD spectra for the system Cu^{II} / Ac-HMAAA, at T = 298.2 K.
$[Cu^{II}]_{tot}$ = 3.0 mM; $[L]_{tot}$ = 3.0 mM.

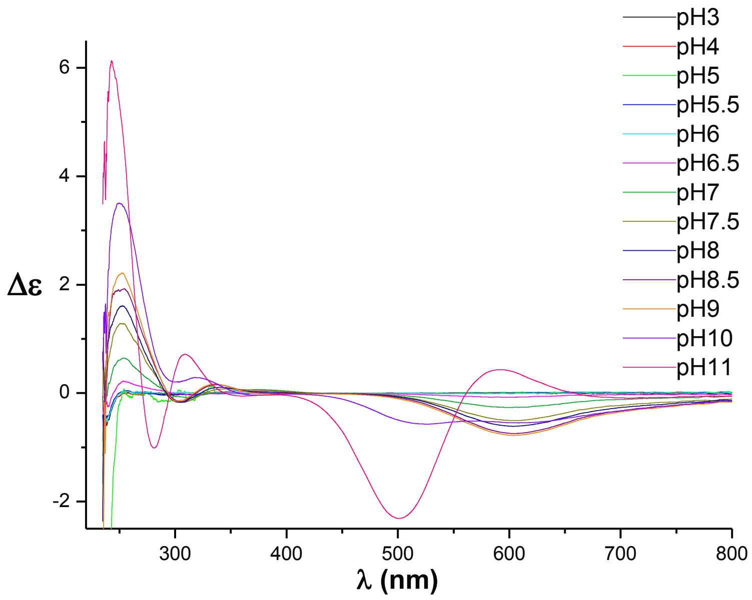

Fig. S9b - EPR spectra for the system Cu^{II} / Ac-HMAAA.
$[Cu^{II}]_{tot}$ = 2.0 mM; $[L]_{tot}$ = 2.0 mM.

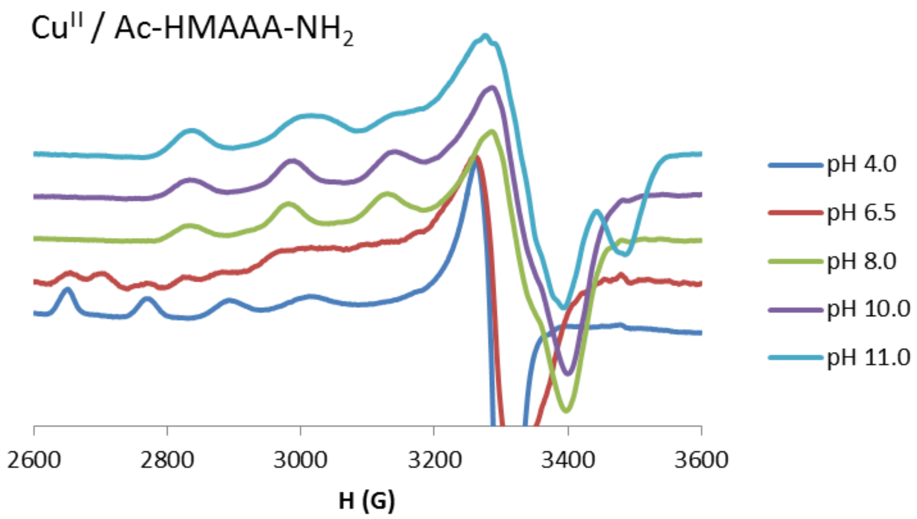

Fig. S10a - CD spectra for the system Cu^{II} / Ac-HAMAA, at *T* = 298.2 K.
$[Cu^{II}]_{tot}$ = 3.0 mM; $[L]_{tot}$ = 3.0 mM.

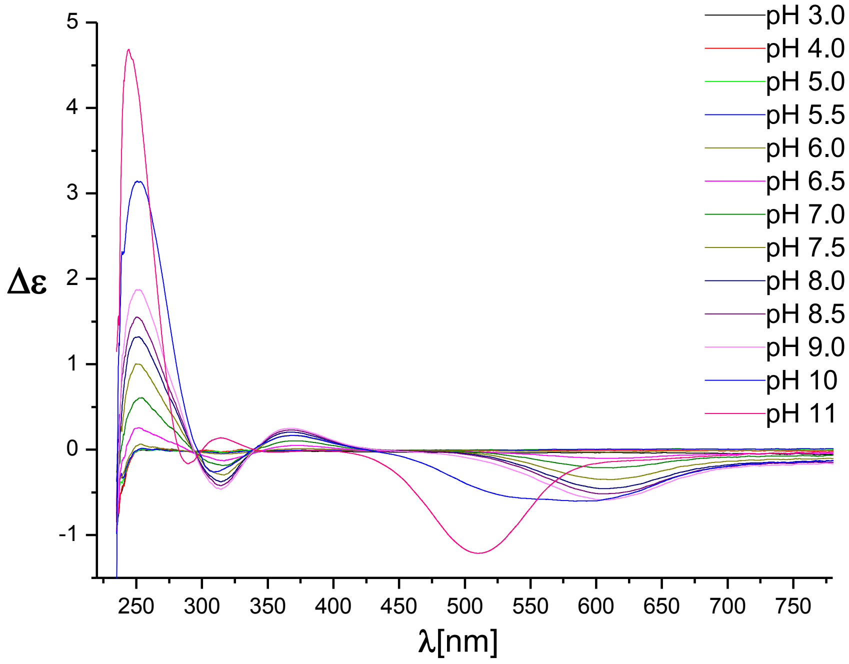

Fig. S10b - EPR spectra for the system Cu^{II} / Ac-HAMAA.
$[Cu^{II}]_{tot}$ = 3.0 mM; $[L]_{tot}$ = 3.0 mM.

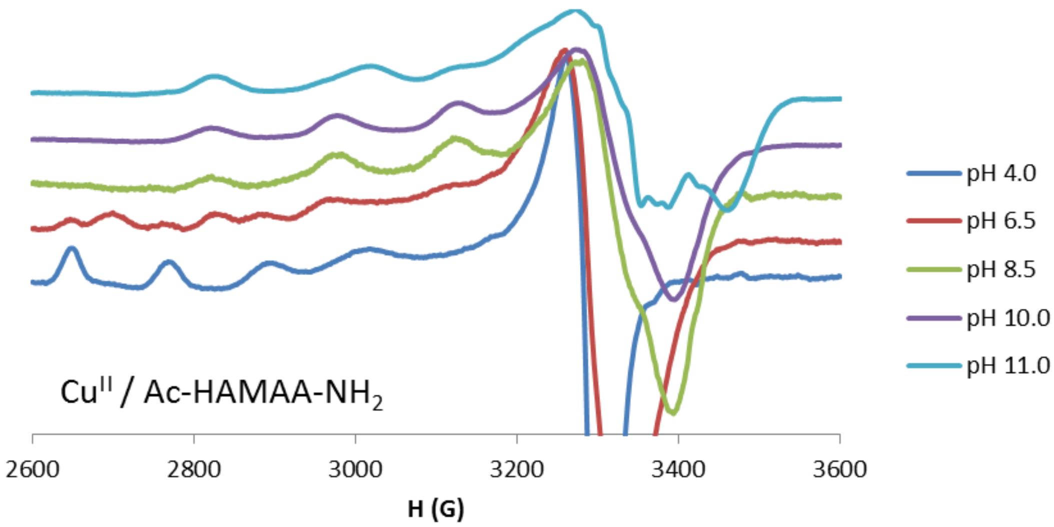

Fig. S11a - CD spectra for the system Cu^{II} / Ac-HAAMA, at *T* = 298.2 K.
$[Cu^{II}]_{tot}$ = 3.0 mM; $[L]_{tot}$ = 3.0 mM.

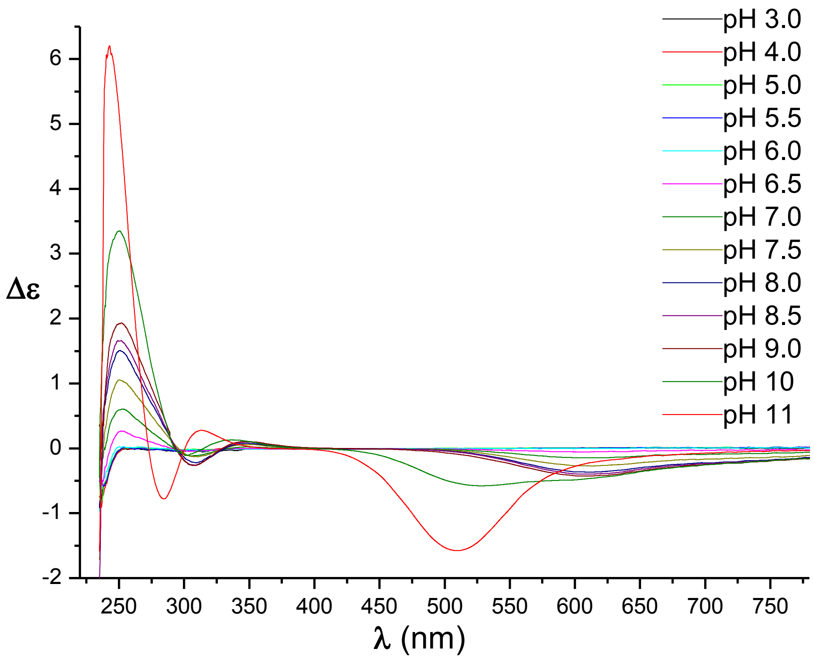

Fig. S11b - EPR spectra for the system Cu^{II} / Ac-HAAMA.
$[Cu^{II}]_{tot}$ = 2.0 mM; $[L]_{tot}$ = 2.0 mM.

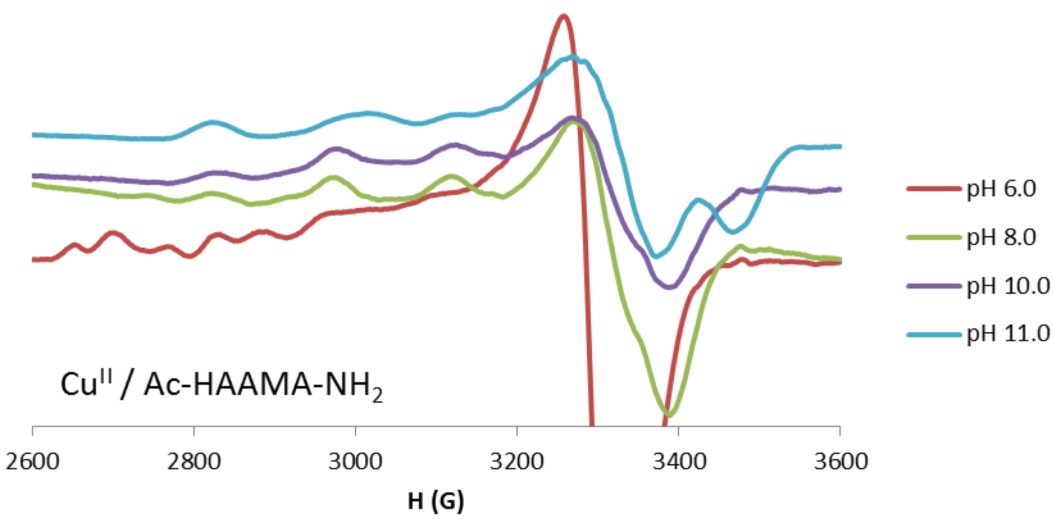

Fig. S12a - CD spectra for the system Cu^{II} / Ac-HAAAM, at T = 298.2 K.
$[Cu^{II}]_{tot}$ = 3.0 mM; $[L]_{tot}$ = 3.0 mM.

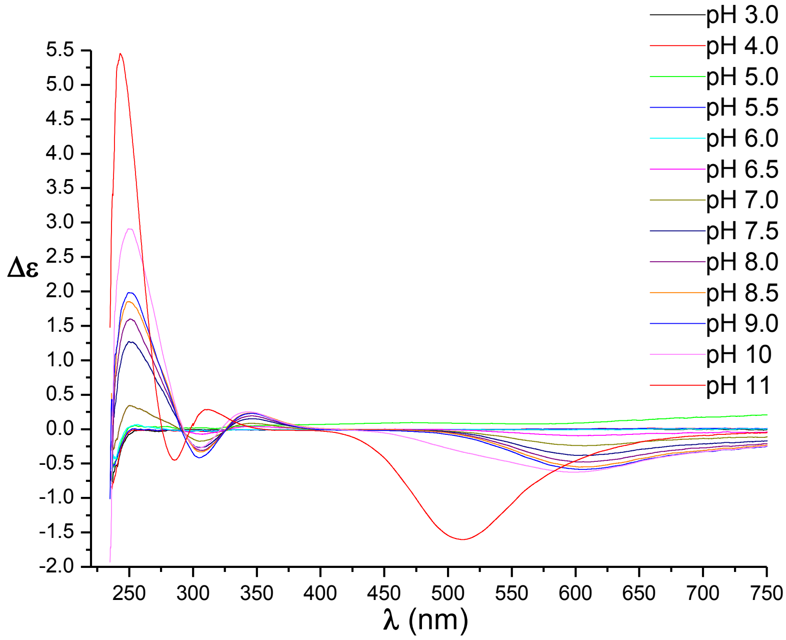

Fig. S12b - EPR spectra for the system Cu^{II} / Ac-HAAAM.
$[Cu^{II}]_{tot}$ = 3.0 mM; $[L]_{tot}$ = 3.0 mM.